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A SERS nano-tag-based fiber-optic strategy for *in situ* immunoassay in unprocessed whole blood

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ABSTRACT

Assay technologies capable of detecting biomarker concentrations in unprocessed whole blood samples are fundamental for applications in medical diagnostics. SERS nano-tags integrated fiber-optic biosensor (FOB) was realized for the first time for *in situ* immunoassay in whole blood. The reliability and sensitivity of this method rely, in a large extent, on the quality and properties of the SERS nano-tags. The constructed silica-coated Ag SERS nano-tags as labels were used in a rapid and specific *in situ* FOB immune sensor to detect alpha fetoprotein (AFP) in unprocessed blood samples. Preliminary results of *in vivo* and *in situ* dynamic observation of AFP of whole blood in wistar rat highlight the power of this new method.

1. Introduction

The detection of the contents of blood composition can provide scientific basis for clinic diagnosis, prognosis, and prevention and control measure. Unfortunately, the relevant detections are mainly carried out in plasma or serum samples, separated from the whole blood, which require considerable sample preparation, making these assays expensive, time-consuming, complexity, less accurate. Worse, this requires that the tests are carried out in the central laboratories and precludes them from most point-of-care (POC) applications (Sun et al., 2013). Thus, for many POC and public health applications, it is very important to design practicable strategy to detect biomarker directly in unprocessed whole blood samples without introducing the complicated sample preparation.

Among the developed detection techniques, optical detection methods have been regarded as one of the most convenient tools due to the simplicity and low detection limit (Pansare et al., 2012; Wencel et al., 2014). However, the strong scattering, absorption, and significant autofluorescence from the whole blood limit their application in the whole blood (Arppe et al., 2015; Pansare et al., 2012; Xie et al., 2013). Some articles have reported homogeneous assay in the application of whole blood, the results of detection would hold many errors and the lower sensitivity because the light scattering effect has to be thought due to the complexity of whole blood compositions according to the light scattering theory (Li et al., 2012; Wencel et al., 2014). The only manner to eliminate the light scattering effect is to avoid the light spread in the whole blood solution. Taking advantage of optical fiber, we introduce the optical fiber bio-sensing technology to eliminate the influence of light scattering effect (Zhang et al., 2010; Wang and Wolfbeis, 2013; Bosch et al., 2007; Leung et al., 2007). Where the function of optical fiber is to transmit the excitation light and signal, and the other function is used as the supporter of the indicators. Among one of them, fiber-optic bio-sensor(FOB) based on evanescent wave has great potential in POC application due to many advantages such as easy operation, no separation, higher sensitivity and the detection can be implemented *in situ,* real-time and *in vivo* (Taitt et al., 2005; Zhang et al., 2010). The evanescent wave from the fiber core can only excites the fluorophores in vicinity of fiber core within about 100 nm, partly avoiding the interference from the environment, bringing the above merits. However, in whole blood assay, the blood can still exist in the evanescent wave region. So, the direct application of this technology into whole blood sample maybe demands that both excitation and detection region lie in the near-infrared (NIR) window to avoid background interference, because the whole blood possesses strong background absorption and autofluorescence in UV–visible (UV–Vis) region (Arppe et al., 2015).

SERS nano-tags, as NIR probes, have gained widespread attention due to their exceptional photophysical properties (Sha et al., 2008;

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Wang et al., 2013). For example, compared to other tags, *e.g*. QDs, and dyes, SERS tags are superior in multiplexing, ultra-sensitivity, high-photostability, and quantitative abilities. Importantly, SERS nano-tags overcome the shortcomings of the other NIR probes, *e.g*. no photo-bleaching and the much more number of distinct signals in the NIR window. Therefore, SERS nano-tags are under more and more active investigation for *in vivo* applications. Moreover, SERS nano-tags have been also employed in homogeneous assay in unprocessed blood (Sha et al., 2008). Therefore, SERS nano-tags in combination with FOB has great potential to be used to develop a simple and *in situ* assay method in whole blood samples without the blood separation steps.

In this work, taking advantage of the intrinsic properties of the SERS nano-tags and FOB, we have developed a SERS nano-tag-based fiber-optic strategy to be successfully used for *in situ* immunoassay in the unprocessed whole blood that overcomes the current assay limitations. This method largely relies on the properties of SERS nano-tags. We have prepared silica-coated SERS nano-tags, comprised of one SERS-active Ag nanoparticle (NP) and a sub-monolayer of 4-MBA Raman molecules adsorbed to the Ag NP surface, and a silica shell to protect SERS nano-tags and further functionalization. The as-constructed Ag@4-MBA@SiO_2 nano-tags displayed stronger SERS signals with the enhancement factor of 1.5×10^7 (AEF), and processed excellent stability in the unprocessed whole blood solutions. We further integrated these Ag@4-MBA@SiO_2 SERS tags with optical fiber to construct an *in situ* immune assay for direct measuring cancer biomarker, alpha-fetoprotein (AFP) in unprocessed whole blood solutions. The results demonstrated the constructed FOB in whole blood held sensitive and reproducible response to the AFP concentration in the range from 50 to 500 ng/mL. Preliminary results of *in vivo* and *in situ* dynamic observation of AFP of whole blood in wistar rat demonstrated this method had the great potential in *in vivo* assay. At the same time, the dynamic observation of AFP in PBS and unprocessed blood samples proved the complicated composition seriously influenced the diffusion coefficient.

2. Experimental section

2.1. Materials

Tetraethyl orthosilicate (TEOS, 98%), aminopropyltriethoxysilane (APTMS) and 4-mercaptobenzoic acid (4-MBA) were purchased from Sigma-Aldrich. Sodium citrate, dimethylamine, anhydrous isopropanol, $NaBH_4$ and NaCl were obtained from Beijing Chemical Plant. Silver nitrate ($AgNO_3$, ≥99%) was purchased from Fluka. The whole blood was supplied by Changchun Blood Supply Center (Changchun, China). Alpha Fetoprotein antibody (Rabbit anti-AFP) and Alpha Fetoprotein (AFP) were purchased from Beijing Dingguo Biotechnology Company (Beijing, China). Deionized water was purified through a Milli-Q water purification system and the resistivity was 18.2 MΩ cm.

2.2. Encapsulating the SERS-Ag NPs with silica

SERS reporter-functionalized Ag NPs were further encapsulated with a silica shell based on our before article (Zhang et al., 2015). Simply, as-prepared SERS-functionalized Ag NPs solution (2 mL) was added into isopropanol (8 mL) solutions. Under shaking, dimethylamine solution (0.2 mL, 30 wt%) was added to the mixed solution, followed by the addition of TEOS in isopropanol (0.6 mL, 10 mM) three times within 6 h after 10 h, the reaction mixture was then centrifuged at 8500 rpm for 15 min and Ag@4MBA@SiO_2 NP precipitate was redispersed into ethanol for further washing. After three times of washing, Ag@4-MBA@SiO_2 NPs were obtained and redispersed into deionized water or ethanol (2 mL) for characterization and further functionalization.

2.3. Modification of Ag@4MBA@SiO_2 SERS nano-tags

2 μL of APTMS was added into the NPs anhydrous ethanol solution and stirred for 12 h (You et al., 2012). Then, the above solution was centrifuged three times by washing with ethanol. The obtained Ag@SiO_2 NPs were dropped into glutaraldehyde (GA) solution (1%). After 2 h, the Ag@SiO_2 NPs were centrifuged three times with water. The remnant was finally dissolved in 10 mM PBS buffer, and AFP was then added for protein immobilized on the surface of the tag NPs overnight and then dipped into 1% (m/m) albumin from bovine serum (BSA) solution for 2 h.

2.4. Preparation of rabbit anti-AFP functionalized fiber-optical ends and in situ assay in the whole blood

The cladding was firstly taken down from the lateral face of the optical fiber and buffered 12.5 cm from the distal end. To prepare rabbit anti-AFP -functionalized optical-fiber was based on our before article (Zhang et al., 2010). Simply, the optical fiber core was firstly cleaned to get rid of the contaminant and activate the hydroxyl. Then, the aldehyde-functionalized fiber probes were obtained by using the reagents: APTMS and GA. At last, the fiber probes were coated overnight with 1 mg/mL rabbit anti-AFP at 4 ℃ and then dipped into 1% (m/m) BSA solution for 2 h. The displacement immunoassay was prepared as follows. Firstly, rabbit anti-AFP modified fibers were added into AFP modified SERS tags solution for 4 h and then cleaned three times.

The FOB was applied to show the advantage of Ag@SiO_2 SERS tag as labels for rapid detection of the AFP in unprocessed whole blood solutions. A series of different concentration of AFP whole-blood solutions were prepared by adding different amount of AFP to 1 mL of whole blood. Then the fibers were dipped into the whole blood samples for 30 min to measure the SERS signals.

In a proof of concept experiment, the AFP was injected into the wistar rat by jugular. After 30 min, the fiber-optical probe was dipped into tail vein by using syringe needle and then measured the SERS signal in different moment in order to demonstrate an *in vivo, in situ* and real time assay concept.

2.5. Characterization

The size of NPs was measured by field emission scanning electron microscopy (FE-SEM, Hitachi, S-4800). The Raman spectra were measured at room temperature by an Ocean Optics QE65000 Raman spectrometer system equipped with a 785 nm laser. All the Raman measurements were completed in the solution.

2.6. Design of direct immunassay in unprocessed whole blood

As talked above, optics-based assay technology is not fit for detecting biomarker directly in whole blood, which is the main subject of strong background absorption in UV–Vis region, autofluorescence and light scattering from the whole blood components.

Here, we design a new strategy of SERS nano-tag integrated fiber-optic sensing stragety for *in situ* detecting biomarker in the unprocessed blood. The SERS nano-tags can be excited by using the NIR laser-785 nm, which clears up the background noise from the whole blood. Thinking about the advantages of the optical fiber, the optical fiber is introduced to eliminate the influence of light scattering from the blood components and even bring the other functions such as *in situ* and *in vivo* by using evanescent wave theory. The reliability and sensitivity of this stragety rely, in a large extent, on the quality and properties of the SERS nano-tags. “Bare” SERS nano-tags are instable and show biotoxicity to limit their applications. Encapsulation of silica on the SERS tags to form core-shell structure is used to overcome the above problems. Fig. 1 shows the basic steps for the unprocessed whole